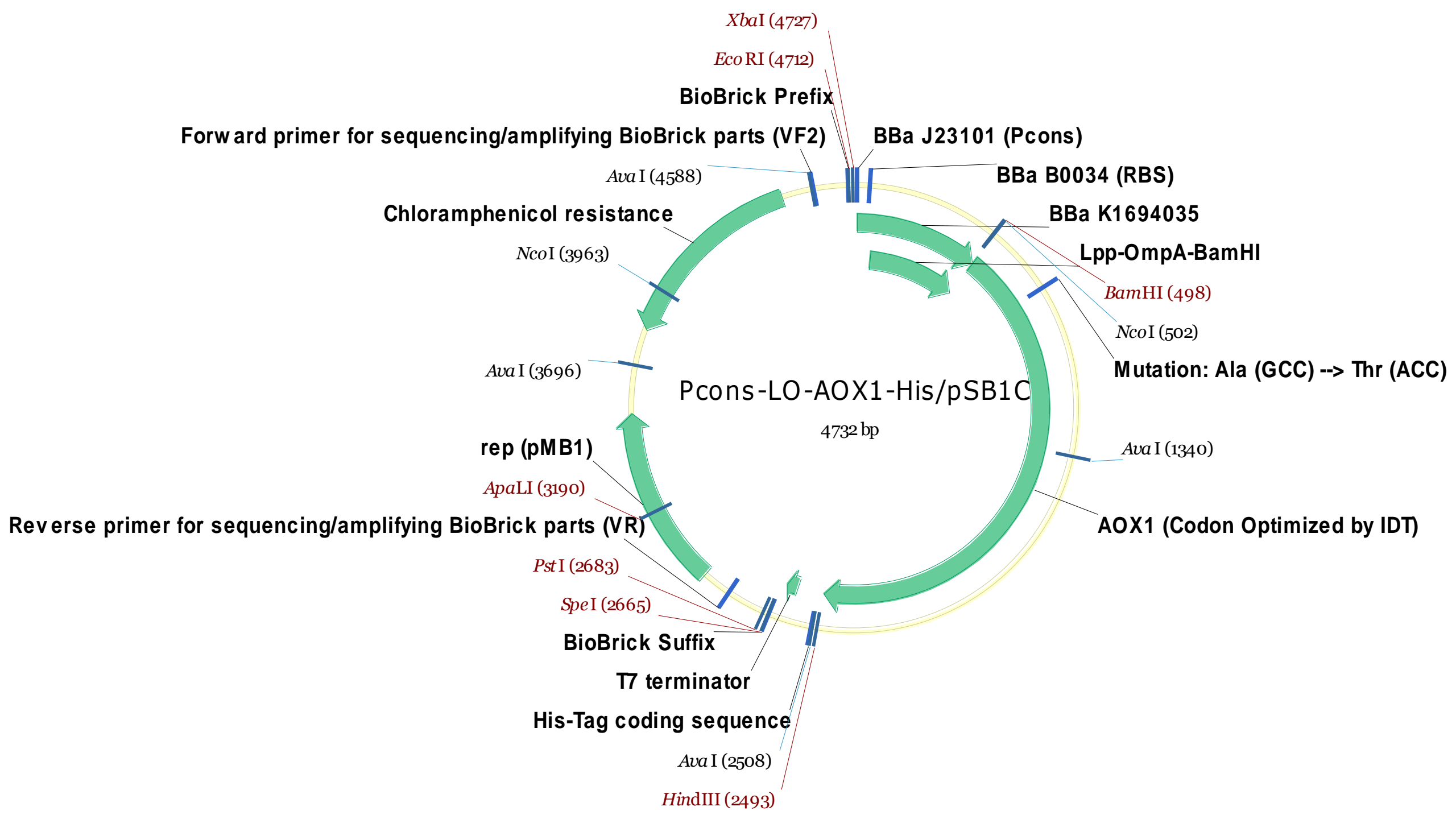


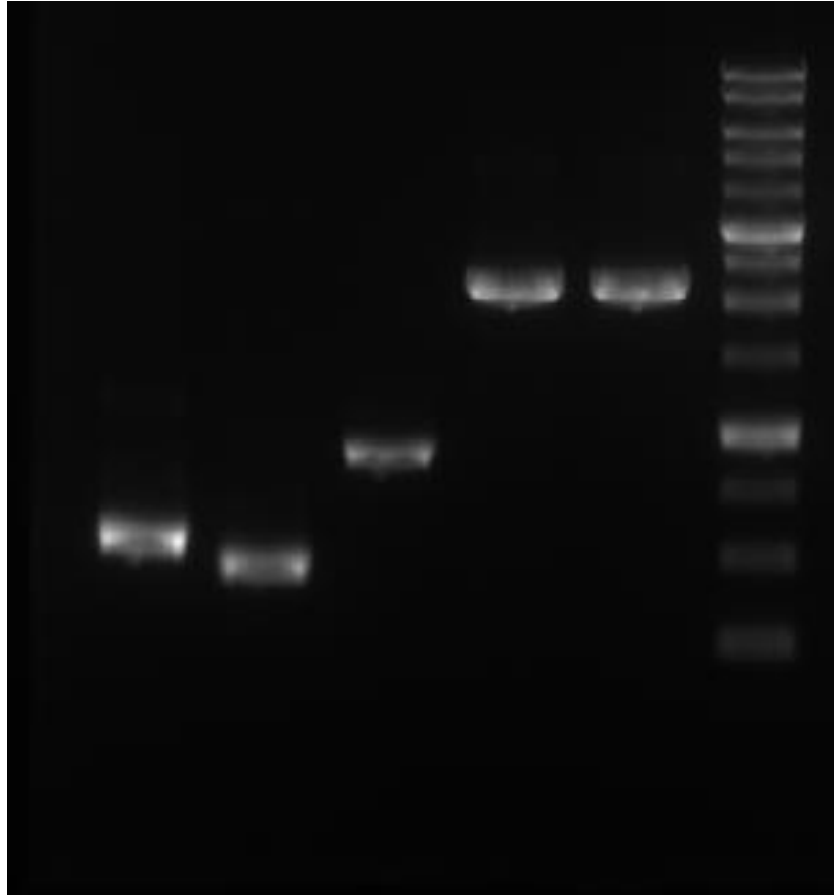
**Pcons-RBS-LO-AOX1-
His/pSB1C3**



Strategy

- The same as “CLONING - LO-AOX1-His_pSB1C3”

PCR with KOD-plus



- Date: 2016.08.17

- Lane:

1. Pcons-RBS-LO-BamHI

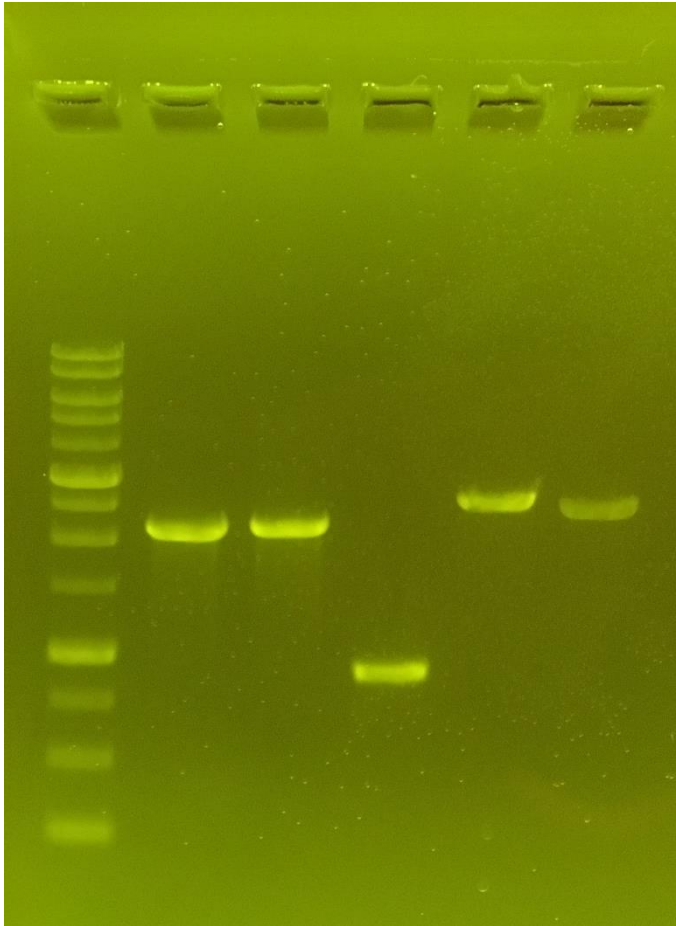
2. LO-BamHI

3. GFP

4. **AOX1**

5. AOX2

Ligation



- Date: 2016.08.31

- Lane:

1. AOX1: BamHI + PstI

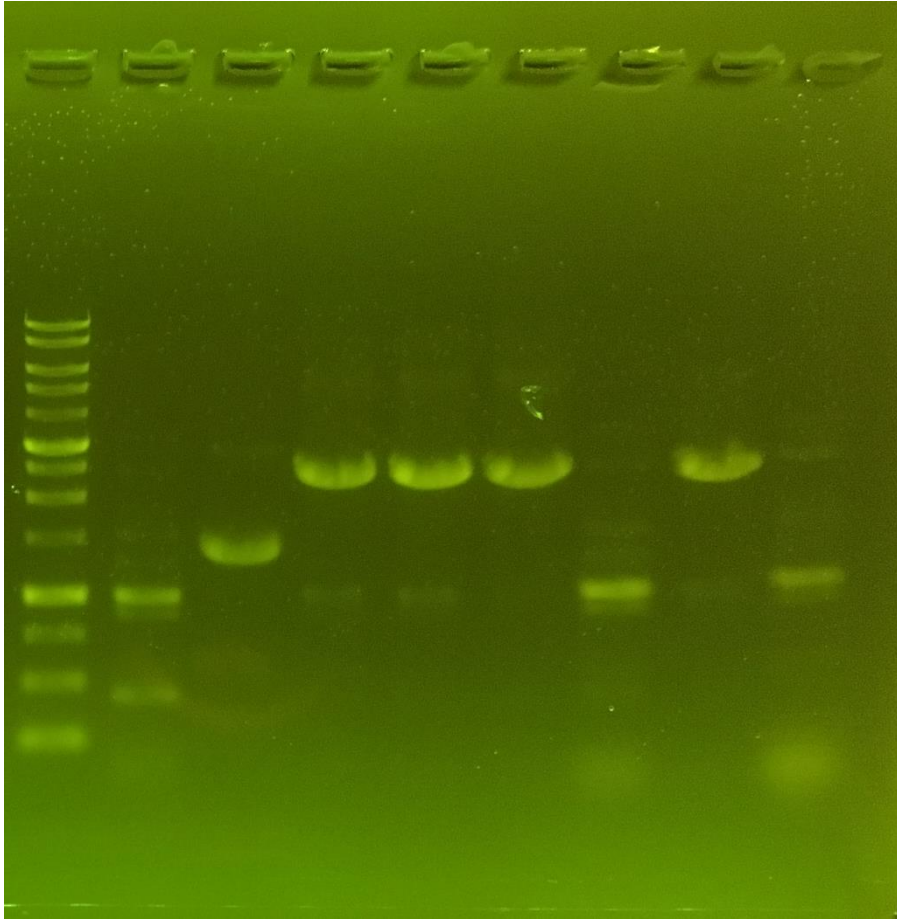
2. AOX2: BamHI + PstI

3. GFP: BamHI + PstI

4. Pcons-RBS-LO-BamHI/pSB1C3
#P6: BamHI + PstI

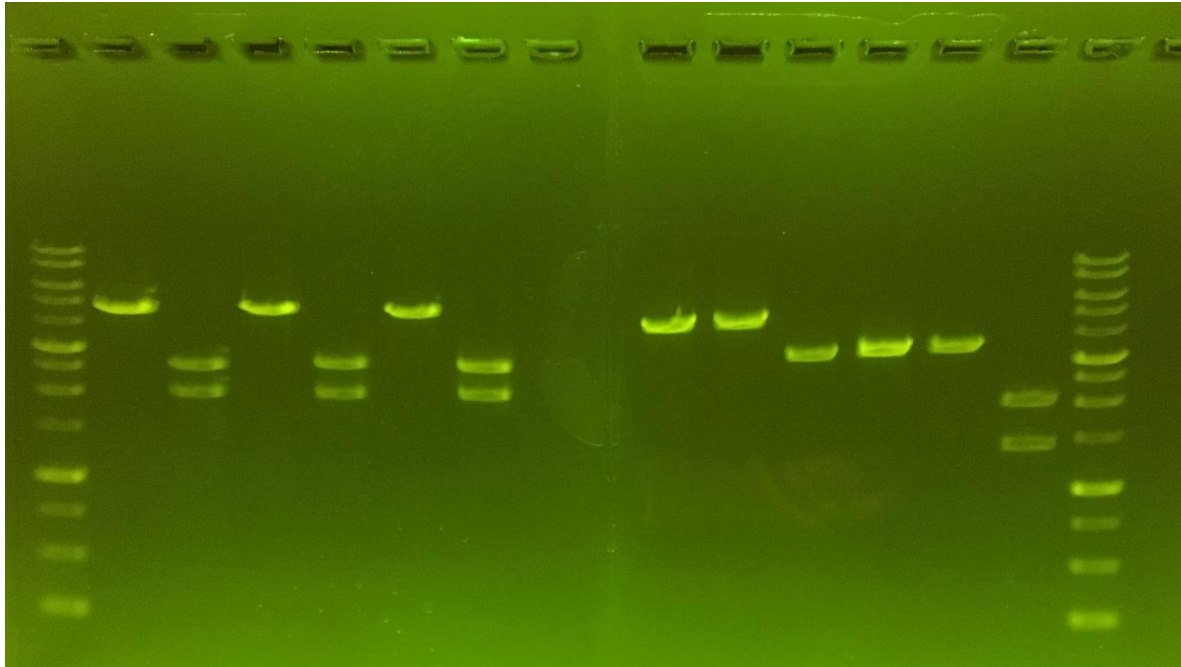
5. LO-BamHI/pSB1C3 #L2: BamHI
+ PstI

Colony PCR



- Date: 2016.09.02
- Clone: Pcons-RBS-LO-AOX1-His/pSB1C3
- Primer:
 1. Pcons-Xbal-EcoRI-F
 2. AOX1-PstI-R
- Lane: #P1-1 ~ #P1-8
- #P1-5 → Plasmid extraction → RE check

RE check



- Date: 2016.09.07
- Clone: LO-GFP-His/pSB1C3
- Lane:
 1. #L1-2: EcoRI
 2. #L1-2: EcoRI + PstI
 3. #P1-5: EcoRI
 4. #P1-5: EcoRI + PstI
 5. #L2-5: EcoRI
 6. #L2-5: EcoRI + PstI
 7. #P2-2: EcoRI
 8. #P2-2: EcoRI + PstI
 9. #LG-3: EcoRI (Failed)
 10. #LG-3: EcoRI + PstI (Failed)
 11. #PG-4: EcoRI
 12. #PG-4: EcoRI + PstI

Sequencing

- Date: 2016.09.14
- Sequencing primers: VF2, VR
- Clone: Pcons-RBS-LO-AOX1-His/pSB1C3 **#P1-5**
- File: “2016.09.14_Pcons-RBS-AOX1_pSB1C3 (#P1-5)”
- Result: revised → make a stock